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Citation for published version (APA):

Bosworth, L.A., Bhaskar, P., O'Brien, M.A., Kriel, H., Smit, E., & Cartmell, S. (2015). Comparison of manufacture and sterilisation techniques on in vivo biological response: a six-week study using electrospun yarns grafted into mouse tendons. In *European cells & Materials* (Vol. 29). AO Research Institute Davos.

Published in:

European cells & Materials

Citing this paper

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Comparison of manufacture and sterilisation techniques on in vivo biological response: a six-week study using electrospun yarns grafted into mouse tendons

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INTRODUCTION: Medical devices intended for implantation into humans require sterilisation using regulatory approved methods. Within a research lab setting however, this sterilisation process often incorporates non-approved techniques because they are considerably cheaper and have a quick turnaround. This study compared the in vivo biological response of electrospun poly(ϵ -caprolactone) fibre yarns fabricated by two different manufacturers and subsequently sterilised by irradiation (approved) or ethanol submersion (non-approved). The efficacy of the implant was further compared to autograft - the current gold standard treatment for tendons requiring reconstruction.

METHODS: Electrospun poly(ϵ -caprolactone) (PCL) yarns were manufactured at two separate sites – in-house (UOM) and The Stellenbosch Nanofiber Company (SNC). Yarns were sterilised by either gamma irradiation (25 kGy, Synergy Health) or submersed in increasing concentrations of ethanol (70-100 % w/v) and washed in phosphate buffered saline solution. Individual yarns were implanted into purpose-made defects within the flexor digitorum longus tendon of mice and held in place by single-knot sutures at either end (Fig. 1A). Yarns were compared to autograft at 3, 21 and 42 days (n=4). Cell infiltration (migration into and along the graft), cell proliferation (cell coverage within implant area) and immunohistochemistry staining for 4 key processes was performed: (1) inflammation (CD45, F4/80, Ly6G); (2) collagen production (Hsp47); (3) cell turnover (BrdU, TUNEL); and (4) blood vessel formation (CD31, SMA). Staining was semi-quantified from serial tissue sections (5 μ m thick) using a maximum +++ or --- score system to indicate the level of positive staining compared to the control.

RESULTS: Cells were observed to have infiltrated all electrospun yarns at 3 days and continued to migrate through to the centre of the implant with time, >90% at 42 days (Fig. 1B).

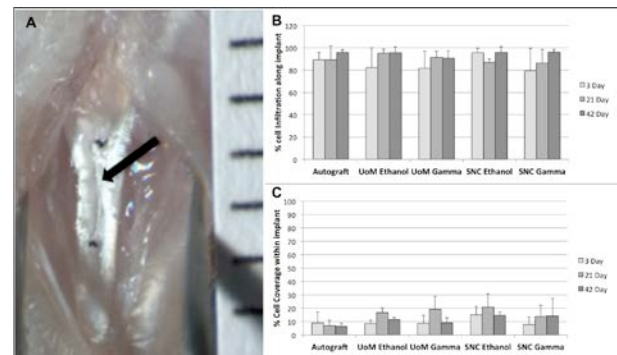


Fig. 1: (A) Arrow indicates position of electrospun yarn in situ. Black dots represent suture knots. (B) % cell infiltration along the implant and (C) % cell coverage within the implant for all electrospun yarns compared to autograft.

Cells colonised a low percentage of the total implant area for all yarns implanted, though cell coverage was comparable to the autograft tissue (Fig. 1C). Collagen production was similar to the autograft control for all yarns over time (Table 1).

Table 1. Level of immunostaining for Hsp47 (collagen production) for implant area compared to autograft (0 = no observed difference).

Hsp47	3 day	21 day	42 day
UOM EtOH	0	0	0
UOM Gamma	0	+	0
SNC EtOH	0	0	0
SNC Gamma	0	0	0

DISCUSSION & CONCLUSIONS: Irrespective of manufacturer and sterilisation technique, the biological response of the electrospun yarns in vivo was similar to the current gold standard, autograft. This would suggest ethanol submersion, as a sterilisation technique is acceptable when undertaking short-term in vivo animal studies.

REFERENCES: ¹ L.A. Bosworth, A. Gibb, S. Downes (2012) *J Polym Sci Part B Polym Phys* 50:870-876.

ACKNOWLEDGEMENTS: This study was supported by the MRC-DPFS (Grant no: G1000788-98812 and MR/M007642/1).